

Claim Listing

Please amend the claims as set forth in the Claim Listing below.

1. (Currently Amended) A mammalian cell-based high throughput assay for the profiling and screening of putative modulators of an epithelial sodium channel (ENaC) comprising: contacting a test cell expressing ~~alpha, beta and gamma subunits or delta, beta and gamma subunits or a variant, fragment or functional equivalent of each of these three subunits, wherein~~ said beta, and gamma subunits respectively have at least 95% sequence identity with the amino acid sequence encoded by SEQ ID NO.: 2, and 3 and the delta subunit has at least 95% sequence identity with the amino acid sequence in SEQ ID NO:8, and said cell is preloaded with a membrane potential fluorescent dye or a sodium fluorescent dye with at least one known ENaC inhibitor under conditions that at least partially inhibits ENaC function and thereafter contacting the test cell with at least one putative modulator compound in the presence of ~~sodium or lithium;~~ and monitoring ~~anion-mediated changes in fluorescence of~~ emitted by the test cell in the presence of the putative modulator/ENaC interactions compared to changes in fluorescence emitted by the test cell in the absence of the putative modulator compound in order to determine the extent of ENaC modulation of the putative ENaC modulator compound and based on said comparison in detected fluorescence determining whether said compound modulates the ENaC channel.

2. (Previously Presented) The method of claim 1 wherein the known ENaC inhibitor is an amiloride derivative.

3. (Previously Presented) The method of claim 2 wherein said compound is selected from the group consisting of Phenamil, benzamil, ethylisopropylamiloride; 2.sup.1, 4.sup.1-dimethylbenzamil (DMB), 5-(N-4-chlorobenzyl)-2.sup.1,4.sup.1-dimethylbenzamil (CBDMB); 3.sup.1,4.sup.1-dichlorobenzamil; 5-(N-methyl-N-guanidinocarbonyl)methyl amiloride, 5-(N,N-hexamethylene)amiloride; 5(N-ethyl-N-isopropyl)amiloride (EIPA); 5-(N-4-chlorobenzyl)-2.sup.1,4.sup.1 dimethylbenzamil; 2.sup.1,4.sup.1-dimethyl 2.sup.1,3.sup.1-benzamil 2.sup.1,3.sup.1-benzobenzamil; and 5-(N-4-chlorobenzyl)-2.sup.1,4.sup.1 dimethylben-zamil.

4. (Previously Presented) The method of claim 3 wherein said compound is Phenamil.

5. (Canceled)

6. (Canceled)

7. (Previously Presented) The assay method of claim 1 in which the test cell is selected from the group consisting of MDCK, HEK293, HEK293 T, BHK, COS, NIH3T3, Swiss3T3 and CHO.

8. (Previously Presented) The assay method of claim 7 in which the cell is an HEK293 cell.

9. (Previously Presented) The assay method of claim 7 wherein said HEK293 cell is an HEK293T cell.

10. (Previously Presented) The assay method of claim 1 in which a said method is used to identify a compound as one which particularly modulates taste based on a detectable change in fluorescence.

11. (Previously Presented) The assay method of claim 10 wherein said taste is salty taste.

12. (Previously Presented) The assay method of claim 1 in which said test cells are seeded onto a well of a multi-well test plate.

13. (Previously Presented) The assay method of claim 12 wherein said test cells are contacted with a putative modulator by adding said putative modulation to the well of said multi-well test plate.

14. (Previously Presented) The assay method of claim 13 wherein said test cells are loaded with a membrane potential dye that allows for changes in fluorescence to be detected.

15. (Canceled)

16. (Canceled)

17. (Canceled)

18. (Canceled)

19. (Canceled)

20. (Previously Presented) The assay method of claim 1, wherein said ENaC subunits all comprise human ENaC subunits cloned from human kidney cDNA.

21. (Previously Presented) The assay method of claim 1, wherein said ENaC subunits comprise human ENaC subunits cloned from human lung cDNA.

22. (Previously Presented) The assay method of claim 1, wherein the ENaC is a human ENaC that is encoded by human ENaC DNA sequences cloned from human taste cell cDNA.

23. (Previously Presented) The assay of claim 1, wherein the ENaC is comprised of alpha (or delta), beta and gamma subunits and selected from the group consisting of: a naturally occurring human ENaC, an alternatively spliced human ENaC, a functional variant thereof, or combinations thereof.

24. (Previously Presented) The assay of claim 1 wherein a fluorescence plate reader is used to monitor changes in fluorescence.

25. (Previously Presented) The assay of claim 1 wherein a voltage imaging plate reader is used to monitor changes in fluorescence.

26. (Previously Presented) The assay of claim 1 wherein the membrane potential dye is selected from the group consisting of Molecular Devices Membrane Potential Kit (cat#R8034), Di-4-ANEPPS (Pyridinium, 4-(2-(6-(dibutylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl))-hydroxide, inner salt), DiSBACC4(2) (bis-(1,2-dibarbituric acid)-trimethine oxanol), DiSBAC4(3) (bis-(1,3-dibarbituric acid)-trimethine oxanol), CC-2-DMPE (Pacific Blue.TM. 1,2-ditradecanoyl-sn-glycerol-3-phosphoethanolamine, triethylammonium salt) and SBFI-AM (1,3-Benzenedicarboxylic acid, 4,4'-[1,4,10-trioxo-7,13-diazacyclopentadecane-7,13-diylbis(5-methoxy-6,1-2-benzofurandiyl)]bis-, tetrakis[(acetyloxy)methyl]ester; (Molecular probes).

27-110. (Canceled)